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=> s (protease or peptidase or proteinase) (4A) (auto or self)
L2 571 (PROTEASE OR PEPTIDASE OR PROTEINASE) (4A) (AUTO OR SELF)

=> s (fusion protein)(4A) (auto or self)
L3 156 (FUSION PROTEIN)(4A) (AUTO OR SELF)

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=> d l5 1-5 bib ab

L5 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2005:277491 CAPLUS
DN 142:458829
TI Self-cleavage of fusion protein in vivo
using TEV protease to yield native protein
AU Shih, Yan-Ping; Wu, Hui-Chung; Hu, Su-Ming; Wang, Ting-Fang; Wang, Andrew
H.-J.
CS Institute of Biological Chemistry and National Core Facilities of High
Throughput Protein Production, Academia Sinica, Taipei, 115, Taiwan
SO Protein Science (2005), 14(4), 936-941
CODEN: PRCIEI; ISSN: 0961-8368
PB Cold Spring Harbor Laboratory Press
DT Journal
LA English
AB Overprodn. of proteins from cloned genes using fusion protein expression
vectors in Escherichia coli and eukaryotic cells has increased the
quantity of protein produced. This approach has been widely used in
producing soluble recombinant proteins for structural and functional anal.
One major disadvantage, however, of applying this approach for clin. or

bioindustrial uses is that proteolytic removal of the fusion carrier is tedious, expensive, and often results in products with addnl. amino acid residues than the native proteins. Here we describe a new method for productions of native proteins with original amino termini in vivo via intracellular self-cleavage of the fusion protein using tobacco etch virus (TEV) protease. Our design allows one to simultaneously clone any gene into multiple fusion protein vectors using two unique cloning sites (i.e., SnaBI and XhoI) without restriction digestion, and then rapidly identifies those constructs producing soluble native proteins. This method will make the fusion protein approach more feasible for protein drug research.
RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2003:875431 CAPLUS
DN 139:359898
TI Reporter-selectable hepatitis C virus replicon and its stably transfected hepatoma cell line for drug screening
IN Duggal, Rohit; Patick, Amy Karen; Zhang, Jie; Zhao, Weidong
PA Pfizer Inc., USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003091439	A1	20031106	WO 2003-IB1687	20030422

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT,

TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
CA 2483513 A1 20031106 CA 2003-2483513 20030422
AU 2003225479 A1 20031110 AU 2003-225479 20030422
EP 1499727 A1 20050126 EP 2003-747188 20030422
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK
JP 2005523705 T 20050811 JP 2003-587967 20030422
MX 2004PA10548 A 20050125 MX 2004-PA10548 20041025
PRAI US 2002-375667P P 20020426
WO 2003-IB1687 W 20030422

AB The invention relates to a reporter-selectable hepatitis C virus (HCV) replicon, and use of the replicon to generate stable, human hepatoma cell lines. Specifically, a replicon (BB7-M4-hRLuc) has been constructed containing the 5' NTR fused to a small portion of the core coding region, the humanized Renilla luciferase gene (hRLuc), a self-cleaving peptide of foot and mouth disease virus (FMDV) 2A proteinase, the NPTII gene, and an EMCV IRES (designated EI), followed by the NS3 to NS5B HCV coding region and the 3' NTR region. The replicon has two adaptive mutations in NS3 (E1202G and T1280I) and one in NS5A (S2197P). The stable hepatoma cell line (BB7M4hRLuc#10) stably transfected with this HCV replicon is capable of generating 700,000 relative light units (RLU, units for expressing luciferase activity) of reporter gene activity, which amts. up to a 70 fold difference in signal to noise ratios compared to other available

stable cell line. The replicon and cell lines are useful in the compound
screening process in HCV drug discovery.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1996:510609 CAPLUS
DN 125:161813
TI Cloning, expression and purification of HIV-1 protease
AU Wan, Min; Takagi, Masahiro
CS Faculty Medicine, National University Singapore, Singapore
SO Microbial Utilization of Renewable Resources (1996), Volume Date 1995, 9,
348-361
CODEN: MURRE6
PB International Center for Cooperative Research in Biotechnology, Japan
DT Journal
LA English
AB Various constructs containing the N-terminal extended human immunodeficiency
virus type 1 (HIV-1) protease gene (PR 107) were studied. The constructs
of PR 107 gene expressed as fusion protein with the glutathione
S-transferase of the GST gene or the maltose-binding protein (MBP) of the
Mal E gene showed that the full-length fusion protein
exhibited self-processing in E. coli. The results from expts.
indicated that the size of the fusion portion could not affect the
self-processing of HIV-1 protease obviously. Although
the larger fusion portion (MBP) may offer bigger steric-interference for
the formation of the appropriate conformation of the fused
protease and therefore lower the self-cleavage process,
the protease could still easily self-process from the
fusion portion to release itself, despite that only one subunit of the
dimeric protease attached to GST or MBP. An isolation method consisting
of denaturation of protein and followed by refolding was developed for
releasing this recombinant HIV-1 PR into the soluble phase since most of the
expressed protease was initially present in insol. inclusion bodies. More
than 600-fold purification was obtained by sequential purification using Sephadex
G-50 gel filtration and CM-23 cellulose cation exchange chromatog.,

yielding the protease whose purity was more than 95%. SDS-PAGE indicated that the mol. weight of this recombinant HIV-1 PR is 11 kDa. The recombinant HIV-1 protease showed proteolytic activity for the synthetic peptide substrate corresponding to the sequence of gag MA/CA and pol p6*/PR junctions. Immuno-blotting indicated that these recombinant HIV-1 protease specifically reacted with HIV-1 protease antisera. The purified enzyme whose specific activity for the heptapeptide SQNYPIV was 848.7 nmol*min-1*mg protease-1 also processed recombinant polyprotein Gag41 as its substrate.

L5 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1995:642290 CAPLUS
DN 123:219557
TI Replicating foamy virus-based vectors directing high level expression of foreign genes
AU Schmidt, Michael; Rethwilm, Axel
CS Institut fuer Virologie und Immunbiologie, Universitaet Wuerzburg, Wuerzburg, 97078, Germany
SO Virology (1995), 210(1), 167-78
CODEN: VIRLAX; ISSN: 0042-6822
PB Academic
DT Journal
LA English
AB Replication-competent retroviral vectors (pFOV-1 to -3 and -7) were constructed on the basis of an infectious human foamy virus mol. clone which has deletions in the U3 region of the long terminal repeat and in the 3' region of the genome, previously identified to be nonessential for virus replication in vitro. The CAT and luciferase indicator genes were expressed as C-terminal fusion proteins to 215 amino acids of the viral Bet protein in the pFOV-1 vector. Introduction of the foot-and-mouth disease 2A protease sequence between the truncated bet coding sequence and the cloning site for the insertion of foreign genes in the pFOV-7 vector

resulted in self-cleaving of the recombinant fusion protein. Alternatively, an internal ribosomal binding site was introduced, allowing expression of authentic foreign protein (pFOV-2 and -3 vectors). DNA fragments derived from the mouse hepatitis virus surface gene up to the length of 1.3 kb were inserted into pFOV-1. The vector constructs gave rise to viruses which were fully infectious in diploid human fibroblasts and recombinant viruses stably expressed high levels of foreign protein indicating that the pFOV vectors may be useful tools to study the effects of proteins of interest at least in tissue culture cells.

L5 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1991:576747 CAPLUS
DN 115:176747
TI Self-cleaving fusion proteins
IN Louis, John M.
PA National Institutes of Health, USA
SO U. S. Pat. Appl., 38 pp. Avail. NTIS Order No. PAT-APPL-6-586 079.
CODEN: XAXXAV
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 586079	A0	19910801	US 1990-586079	19900921
US 6077694	A	20000620		
WO 9205276	A1	19920402	WO 1991-US6735	19910920
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
AU 9185377	A	19920415	AU 1991-85377	19910920
PRAI US 1990-586079	A	19900921		
WO 1991-US6735	A	19910920		

AB Self-processing fusion proteins are used for the manufacture of a protein of interest. The fusion protein has three domains: an affinity domain for rapid purification of the protein by affinity chromatog.; a retroviral

proteinase domain flanked by cleavage sites recognized by the proteinase;
and, a domain of interest (the target protein). After purification of the
protein by affinity chromatog. the protein is partially denatured allowing
the proteinase to cleave it into three domains. When the fusion protein
is accumulated as inclusion bodies, it may be cleaved during the
denaturation/solubilization process. Use of sequences from the pol region
of the retrovirus gene can be used to control solubility of the protein. The
use of this procedure to purify and accurately cleave soluble and insol.
fusion proteins of human immunodeficiency virus proteinase and the
Escherichia coli malE gene product was demonstrated.

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1039	protease near4 (fusion protein)	US-PGPUB; USPAT	ADJ	OFF	2007/12/20 20:10
L2	83	protease near4 (auto)	US-PGPUB; USPAT	ADJ	OFF	2007/12/20 20:11
L3	6	l1 and l2	US-PGPUB; USPAT	ADJ	OFF	2007/12/20 20:11

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